RESEARCH

# Expansion of PCR-based Marker Resources in Oat by Surveying Genome-Derived SSR Markers from Barley and Wheat

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**ABSTRACT**

Identifying polymerase chain reaction (PCR)-based markers in crop genomes and amplifying them with specific primer pairs has provided convenient molecular markers for mapping projects. Oat (*Avena sativa* L.) lags behind other crops in the utilization of PCR-based markers due to limited development of genomic and genetic resources in *Avena* species. We surveyed 356 genome-derived simple sequence repeat (SSR) markers from wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), chosen on the basis of even dispersal across different chromosomes, to search for an alternate method of expanding the PCR-based marker pool in oat. Primer pairs for these SSR markers were tested for amplification and polymorphism between parental lines from Ogle1040/TAM-O-301 (OT) and Kanota/Ogle157 (KO) mapping populations. Eighty-nine of 210 wheat primer pairs (42%) and 56 of 146 barley primer pairs (38%) successfully amplified sequences in oat. Forty-five percent of the amplified markers, representing 19% of the total markers, showed polymorphism between parental lines of at least one mapping population. The polymorphism was primarily the presence or absence of a product band. Fifteen PCR products from 10 primer pairs were tested for reproducibility by amplifying each marker in the OT population. When assayed with the same PCR conditions used in the survey, the segregation ratio of 14 markers did not differ from the 1:1 ratio expected for a single locus. This study indicates that genomic SSR primer pairs from wheat and barley may be a good way to efficiently generate PCR-based DNA markers for oat genetics research.

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**Abbreviations:** CTAB, cetyl trimethyl ammonium bromide; EST, expressed sequence tag; KO, Kanota × Ogle157; OT, Ogle1040 × TAM-O-301; PCR, polymerase chain reaction; RIL, recombinant inbred line; SSR, simple sequence repeat.

Simple sequence repeats (SSRs), or microsatellites, are abundant in plant genomes (Wang et al., 1994) and variations of the repeat number among different lines within a species occur at high frequency. These characteristics, along with an evenly dispersed genomic distribution, are properties which make SSRs ideal genetic markers (Morgante and Olivieri, 1993; Powell et al., 1996). In addition, the convenience and rapid assays accompanying SSR markers make them useful for a wide range of genetic and genomic applications (McCouch et al., 2002; Röder et al., 1995; Varshney et al., 2005). In oat (*Avena sativa* L.) there are currently few polymerase chain reaction (PCR)-based markers, including SSR types, available due to limited genomic sequence information.

The SSR markers are popular in species that are closely related to oat. In wheat (*Triticum aestivum* L.), over 2000 SSRs have been mapped (Eujayl et al., 2002; Gupta et al., 2002; Paillard et al., 2003; Pestsova et al., 2000; Röder et al., 1998; Somers et al., 2004; Song et al., 2005; Torada et al., 2006; Yu et al., 2004). La Rota et al. (2005) summarized over 9000 potential SSR sequences identified in wheat. Based on this information, it is expected that high density wheat SSR maps will be developed in the future. Similarly, over 400 SSR markers have been mapped in barley (*Hordeum vulgare* L.) (Li et al., 2003; Ramsay et al., 2000) and approximately

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5000 additional candidate SSR markers exist (La Rota et al., 2005). In contrast, a small number of SSR markers have been mapped in oat (Jannink and Gardner, 2005; Li et al., 2000). A major disadvantage for identifying SSRs in oat compared to wheat and barley is the lack of genetic information available. This makes the development of SSR markers from the oat genome difficult.

A possible way to generate PCR-based markers in oat would be to use SSR primer pairs from related species such as barley and wheat. Jones et al. (2001) tested the amplification efficiency of SSR primer pairs from ryegrass (*Lolium perenne* L.) and found that 12% amplified in oat species. Li et al. (2000) tested 54 SSR primer pairs from barley on 12 wild oat species and 20 oat cultivars. Twenty-six percent (14 of 54) amplified distinguishable bands using an annealing temperature of 53°C. Gupta et al. (2003) reported that 24 of 59 (40.7%) wheat expressed sequence tag (EST)-SSR primer pairs successfully amplified fragments in five species, including oat. The objective of this study was to test the feasibility of using genomic SSR primer pairs from barley and wheat for large scale development of PCR-based marker in oat.

# MATERIALS AND METHODS

## DNA Extraction

Seed of TAM-O-301, Ogle 1040, Kanota156, Ogle157 (Holland et al., 2001; Portyanko et al., 2001), and the 136 F6:10 Ogle/TAM-O-301 (OT) recombinant inbred lines (RIL) (Portyanko et al., 2001), were germinated on moistened filter paper in petri dishes incubated in a dark cabinet. After 5 d, approximately four coleoptiles per genotype were harvested and DNA was extracted using a cetyl trimethyl ammonium bromide (CTAB) protocol. In brief, each sample was frozen with liquid N and ground in a 2.0-mL eppendorf tube (Sigma, St. Louis, MO) with a sterile plastic pestle. Five hundred microliters of DNA extraction buffer (containing 140 mmol $L^{-1}$ sorbitol, 220 mmol $L^{-1}$ Tris, 22 mmol $L^{-1}$ EDTA, 800 mmol $L^{-1}$ NaCl, 0.8% CTAB, and 1.0% Sarcosine) was added to each tube and after incubation at 65°C, 300 μL of chloroform/isoamyl alcohol (24:1) was added. The solution was gently mixed, then centrifuged at 6000 *g* for 25 min. The resulting supernatant was added to an equal volume of chilled 70% isopropyl alcohol to precipitate the DNA. Using a sterile pipette tip, the precipitant was removed, washed with 70% ethanol, dried overnight, and resuspended in TE/RNase (1 μg $mL^{-1}$ DNase-free RNase) buffer.

## PCR Amplifications and Scoring

Polymerase chain reaction amplifications were set up in a 96-well format. Each 25-μL reaction contained 60 ng of template DNA, 1 μL of each primer (10 μmol $L^{-1}$), 2.5 μL of 10× buffer, 1 μL of dNTPs with 2.5 mmol $L^{-1}$ concentration for each nucleotide, and 1 unit of *Taq* polymerase (RedTaq, Sigma). The PCR program was 94°C for 3 min followed by 39 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, followed by a 4°C hold. Polymerase chain reaction products were analyzed on 6% nondenaturing polyacrylamide gels stained with ethidium bromide. Polymerase chain reaction amplification products that were consistent in two repeated PCR amplifications were scored for polymorphisms. The sizes of PCR products in oat were estimated using a DNA ladder (100-bp ladder, Bio-Rad, Hercules, CA) in the same gel.

## SSR Primer Set Selection

We selected a244 d fod2.1(l)-28.7(i)-259 ( t)-24.2 TcN0nt was ad

**Table 1. Comparison of oat amplification frequencies using barley and wheat simple sequence repeat (SSR) primers selected from different chromosomes.**

| SSR marker source | | CH1† | CH2 | CH 3 | CH 4 | CH 5 | CH 6 | CH 7 | Mean |
|---|---|---|---|---|---|---|---|---|---|
| Barley | Amplified‡ | 7/19 | 13/34 | 7/16 | 9/20 | 6/19 | 10/18 | 3/20 | 55/146 |
| | % | 37 | 38 | 44 | 45 | 32 | 56 | 15 | 38 |
| Wheat A | Amplified | 3/10 | 6/10 | 6/10 | 4/10 | 7/10 | 4/10 | 5/10 | 35/70 |
| | % | 30 | 60 | 60 | 40 | 70 | 40 | 50 | 50 |
| Wheat B | Amplified | 2/10 | 7/10 | 5/10 | 6/10 | 3/10 | 5/10 | 3/10 | 31/70 |
| | % | 20 | 70 | 50 | 60 | 30 | 40 | 30 | 44 |
| Wheat D | Amplified | 2/10 | 3/10 | 4/10 | 6/10 | 5/10 | 2/10 | 2/10 | 24/70 |
| | % | 20 | 30 | 40 | 60 | 50 | 20 | 20 | 34 |

†CH represents the chromosome and the number following represents the chromosome number. The chromosome number equals the corresponding number in the specific species. For example, CH1 = barley (1H) and chromosome 1 in the A, B, and D wheat subgenomes.

‡Number of SSRs whose primers yielded PCR products in oat/number of SSRs tested.

cM genetic distance (from position 5 to position 48 in the map from Ramsay et al., 2000) amplified in oat. In contrast, six out of nine primer pairs between HvXan and EBmag0793 covering about the same distance (from position 54 to position 95) on the long arm of chromosome 2 amplified in oat (Fig. 1). Although these results suggest that certain locations in the barley and wheat genomes are more likely to provide markers applicable to oat, more markers must be tested to confirm this hypothesis.

## Amplification Profiles of SSR Primer Pairs in Different Genomes

Differential amplification profiles from the same SSR primer pairs across species may reflect differences in the

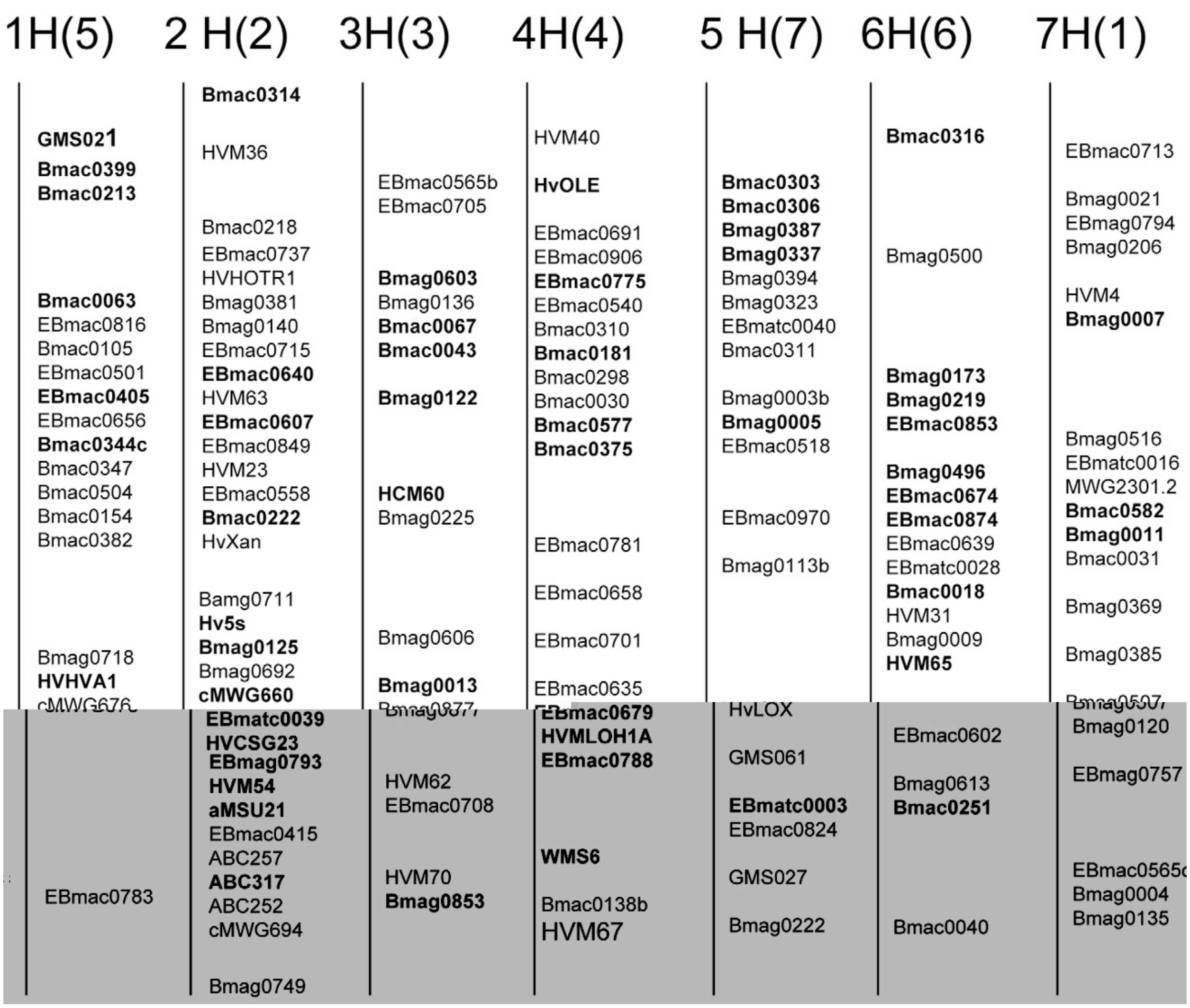


Figure 1. Distribution of simple sequence repeat (SSR) primers selected from barley. Distances between markers do not reflect the exact map positions. Primer pairs in bold letters produced positive polymerase chain reaction (PCR) products in oat lines.

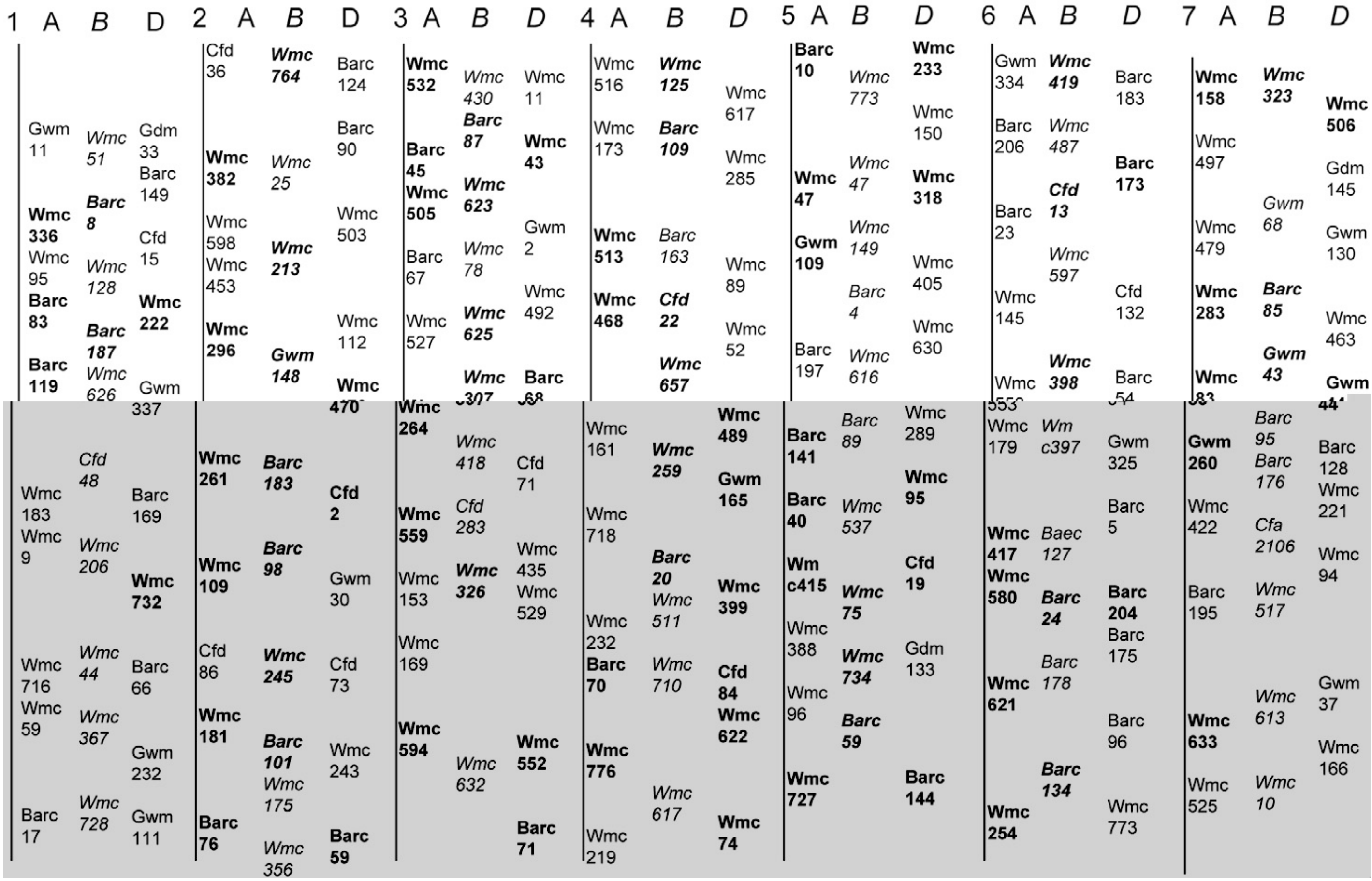


Figure 2. Distribution of simple sequence repeat (SSR) primers selected from wheat. Distances between markers do not reflect the exact map positions. Primer pairs in bold letters produced positive polymerase chain reaction (PCR) products in oat lines. Numbers at the top represent the specific chromosomes. A, B, and D after each number designate the different subgenomes of the chromosomes. Marker Barc59 is indicated on both chromosomes 5B and 2D.

homology of the specific region amplified as well as general complexities of the genomes. To compare the amplification of the same SSR primer pairs in different species, we used SSR primer pairs from wheat chromosome 2 to survey amplification in barley and oat. The amplification profiles for those markers in wheat are based on the Chinese Spring data from GrainGenes (http://www.graingenes.org). The wheat marker profiles were used as a reference and not a direct comparison, because they are not from the same PCR conditions as we used in barley and oat. The cultivars Harrington (Harvey and Rossnagel, 1984) and TAM-O-301 were selected to represent barley and oat, respectively. Twenty-two of 30 wheat SSR primer pairs amplified in barley and 16 amplified in oat. The average number of detected PCR products per primer set was 2.0 in wheat (at 54–61°C annealing temperature), 2.2 in oat, and 1.6 in barley (Table 2). The greater number of PCR products per marker detected in oat and wheat is in line with their polyploidy and larger genome sizes compared to barley.

At the subgenome level in this experiment, SSR primers from wheat chromosome 2 of genome D had the lowest amplification frequency in both barley and oat compared to the primers from chromosome 2 of genomes A and B (Table 2). This result is consistent with the total marker survey results (Table 1), indicating that wheat genome D may be less homoeologous to barley and oat genomes than are wheat genomes A and B.

## Amplification and Relationship between the Repeat Sequences of SSR Markers

To determine if there was a relationship between motif sequence in the donor species SSRs and whether the primer set produced amplification in oat, we summarized the SSR sequence composition in the selected markers and calculated the percentage of each sequence type producing amplified products in oat. Results were derived from 132 of the 146 barley markers because motif information for 14 markers was unavailable. Barley primer pairs most frequently producing amplification in oat were those targeting the motifs AC/TG, AG/TC, CT/GA, and CA/GT (Table 3). These targeted motifs accounted for 89% (117/132) of the total, which was expected since they were the most tested. Motif information was available for 186 of 210 wheat SSR markers. Primer pairs most frequently producing amplification in oat were CA/GT, CT/GA, AC/TG, and ATT/TAA, accounting for 91% (169/186) of the total (Table 3). The primer pairs for the

**Table 2. Comparison of amplification profiles in barley and oat of selected wheat simple sequence repeat (SSR) primers on 3% SFR agarose gel.**

| SSR marker source | Wheat primer set† | | | Amplified in barley | | | Amplified in oat | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of primer pairs | PCR products | PCR products/ primer set | No. of Primer pairs | PCR products | PCR products/ primer set | No. of primer pairs | PCR products | PCR products/ primer set |
| Wheat 2A | 10 | 17 | 1.7 | 8 | 16 | 2.0 | 6 | 17 | 2.8 |
| Wheat 2B | 10 | 18 | 1.8 | 8 | 13 | 1.6 | 7 | 13 | 1.9 |
| Wheat 2D | 10 | 26 | 2.6 | 6 | 7 | 1.2 | 3 | 5 | 1.7 |
| Total | 30 | 61 | 2.0 | 22 | 36 | 1.6 | 16 | 35 | 2.2 |

†Profiles for wheat based on the Chinese Spring deletion mapping data from GrainGenes (http://www.graingenes.org). The polymerase chain reaction (PCR) products were from the optimized PCR conditions for each primer set. The wheat profile is presented as a general reference and not necessarily for direct comparison to barley and oat.

motif CT/GA from both barley and wheat showed a high frequency of PCR amplification in oat. However, primer pairs amplifying barley markers with the AC/TG repeat sequence showed a high amplification frequency (41%) in oat whereas those for wheat SSRs with the same motif showed no amplification in oat (Table 3). This result indicates that there is no useable correlation between the motif of the marker in the donor and amplification in oat.

## Polymorphic Profiles in Oat Detected with Barley and Wheat SSR Primers

The direct usefulness of this survey is the detection of mappable PCR-based markers in oat. Of the 145 barley and wheat primer pairs that amplified products in oat, 65 were polymorphic between parental lines of one or both oat mapping populations (Table 4). The frequencies of product polymorphism in oat using barley and wheat SSR primer pairs were similar with the exception that the polymorphism frequency of the products from wheat D genome primers sets was slightly lower.

Of the 65 primer pairs identifying polymorphic markers in oat, 57 detected polymorphisms between TAM-O-301 and Ogle1040 and 48 detected polymorphisms between Kanota156 and Ogle157 (Table 5). Twenty-five of these primer pairs detected the same polymorphisms between parental lines of the two mapping populations and 11 showed different polymorphic products in two pairs of parental lines. Nineteen primer pairs detected polymorphisms only in the parental pair TAM-O-301 and Ogle1040, while 11 detected polymorphisms only in the parental pair Kanota156 and Ogle157. The majority of the SSR products based on the selected primer pairs ranged from 100 to 200 bp in barley and wheat, whereas the amplification products in oat ranged from 80 to 700 bp (Table 5). Sequencing data of PCR products from oat will be required to confirm if they actually contain SSR sequences.

Since polymorphic patterns of markers are important for mapping and marker-assisted selection, we evaluated the polymorphisms for all the markers. Between TAM-O-301 and Ogle1040, 44 of 55 (80%) were obvious dominant markers. These dominant polymorphic patterns include complete presence and/or absence of PCR products in two parental lines and presence of extra PCR fragments in one line. In the pair Kanota156 and Ogle157, 38 of 46 (63%) were obvious dominant markers. The rest of the markers will be tested for their dominance in the respective mapping population. Overall, most of the polymorphic PCR products were dominant markers in oat.

A high level of polymorphism was detected between Ogle1040 and Ogle157. Seventeen

**Table 3. Distribution of motif sequences in selected barley and wheat simple sequence repeat (SSR) markers and amplification in oat by their primers.**

| Repeat sequences | Barley SSR markers | | | Wheat SSR markers | | |
|---|---|---|---|---|---|---|
| | Tested† | Amplified in oat | Amplification % | Tested | Amplified in oat | Amplification % |
| AC/TG | 39 | 16 | 41 | 21 | 0 | 0 |
| AG/TC | 36 | 14 | 39 | 7 | 1 | 14 |
| AT/TA | 5 | 0 | 0 | 2 | 1 | 50 |
| CA/GT | 15 | 3 | 20 | 85 | 36 | 42 |
| CT/GA | 27 | 11 | 41 | 43 | 22 | 51 |
| ACC | 2 | 2 | 100 | 0 | 0 | 0 |
| ATC/TAG | 4 | 1 | 25 | 1 | 1 | 100 |
| ATT/TAA | 0 | 0 | 0 | 20 | 7 | 35 |
| CAA | 1 | 0 | 0 | 0 | 0 | 0 |
| CCG/GGC | 0 | 0 | 0 | 2 | 0 | 0 |
| GAA | 0 | 0 | 0 | 1 | 0 | 0 |
| GCC | 1 | 1 | 10 | 1 | 1 | 100 |
| TCA | 0 | 0 | 0 | 1 | 0 | 0 |
| TTA | 0 | 0 | 0 | 1 | 0 | 0 |
| ATCT | 1 | 0 | 0 | 1 | 1 | 100 |
| CACT | 0 | 0 | 0 | 1 | 0 | 0 |
| CCCA | 0 | 0 | 0 | 1 | 0 | 0 |
| GATA | 0 | 0 | 0 | 1 | 0 | 0 |
| TAGA | 0 | 0 | 0 | 4 | 2 | 50 |
| TATC | 1 | 0 | 0 | 2 | 2 | 100 |
| TTAA | 0 | 0 | 0 | 1 | 0 | 0 |
| Total | 132 | 48 | 36 | 186 | 75 | 40 |

†Number of markers containing specific repeat sequences. The repeat sequence for each marker was obtained from the GrainGenes database (http://www.graingenes.org). Markers without available repeat sequence information in the database are not included.

primer pairs produced different PCR profiles between these two Ogle lines. Some primers produced additional fragments in one of the two lines. For example, Bmac213 showed an extra 400-bp fragment in Ogle1040 in addition to the 290-bp common fragment (Table 5). Other primer pairs, such as Bmag375, produced totally different amplification profiles in the two Ogle lines. The amplification profile of Bmag375 shows three fragments of 150, 280, and 300 bp in Ogle1040, but two fragments of 500 and 700 bp in Ogle157. Such results, with 26% (17/66) of primers producing polymorphisms in the two Ogle lines, may reflect either the highly complex nature of the oat genome or heterogeneity in the original cultivar. Our results support those of Fox et al. (2001), who found 10 of 66 restriction fragment length polymorphism banding patterns differed between single plant selections from Ogle. They concluded that genetic variability within the original cultivar was responsible for the differences.

## Testing the Polymorphic PCR Products in the Oat Mapping Population under the Screening PCR Conditions

The 50°C annealing temperature in the marker screening experiment is lower than the optimized annealing conditions for most SSR primer pairs in the species from which they originated. To test the reproducibility of PCR products produced in this study, 10 primer pairs that produced polymorphic markers between TAM-O-301 and Ogle1040 were randomly selected for testing on the OT RIL population (Table 6). Among the 15 PCR products tested, 14 segregated in a 1:1 ratio as did the control marker AM112 (Table 6). Three of the PCR products were codominant markers. Thus, most of the polymorphic PCR products derived from barley and wheat SSR primers sets are useful markers that could be mapped in the same way as the oat sequence-derived PCR markers.

## DISCUSSION

In this study, 38% of the SSR primer pairs from barley and 43% of those from wheat successfully amplified products in four oat lines. Of those that amplified in oat, 46% yielded polymorphic markers between parental oat lines used by previous workers to generate mapping populations. This polymorphism rate is comparable to that found with SSR markers derived from oat sequences. In this study, we used acrylamide gel separation, which provided higher resolution than agarose gel separation. Li et al. (2000) reported that 36% of 44 primer pairs designed from oat sequences detected polymorphisms in 20 oat cultivars. Pal et al. (2002) identified 44 SSR markers from oat genomic sequences. Thirteen of the 44 SSR markers (14%) detected polymorphisms between oat lines of Kanota and Ogle. It is understandable that polymorphism detection rates are independent of marker sources as long as sequences are amplifiable. This result is especially important because the limited genomic sequence database in oat has been an obstacle to the development of PCR-based markers in the crop. Oat genetic maps (Portyanko et al., 2001; Wight et al., 2003) are far from ideal largely because of a lack of reliable PCR-based markers. The availability of large pools of SSR primer pairs from wheat and barley (La Rota et al., 2005) should provide a new resource to rapidly develop markers for oat map improvement. With the use of high throughput PCR methods, a 40% amplification frequency is not problematic when using such alternative genetic resources.

Table 4. Number of barley and wheat simple sequence repeat (SSR) markers whose primer pairs amplify products in oat and number and percent producing polymorphic oat markers between parental lines of two oat mapping populations.

| Genome | Amplified | Polymorphic | Polymorphic % |
|---|---|---|---|
| Barley | 55 | 27 | 49 |
| Wheat A | 35 | 14 | 40 |
| Wheat B | 31 | 17 | 55 |
| Wheat D | 24 | 8 | 33 |
| Whole wheat genome | 90 | 39 | 43 |
| Total | 145 | 65† | 46 |

†Barc59 has two loci, one on wheat chromosome 5B and one on 2D. The polymorphic percentage calculations for the subgenome B and subgenome D both counted Barc59, but the in the total number of markers Barc59 was counted only once.

The majority of SSR primer pairs in our study yielded good quality PCR products that were easily scored. In addition to those products, there were also some weakly amplified fragments (Fig. 3). Most of this background amplification was likely due to the generalized PCR parameters. It was necessary to use a single PCR condition for all the primer pairs in the survey due to the large number of primers to be tested. It was thus not practical to optimize amplification conditions for each primer in the survey, but such optimization will be possible in subsequent work aimed at mapping these markers. Another important discovery in this study is that the polymorphic PCR products scored at 50°C annealing temperature are mappable under the same PCR condition (Table 6). Detection and mapping of the PCR products at the same lower annealing temperature as that used in screening may be a good approach to enrich PCR-based markers in oat because the relaxed PCR condition allows partially homologous primers to amplify products. After sequencing such products, primers could be redesigned to create better PCR-based markers for oat.

In comparisons of SSR primer set amplification efficiency across species, among the relatively limited number of primer pairs tested in this study, 73% (22/30) of the wheat SSR primer pairs amplified in barley and 53% (16/30) amplified in oat (Table 2). The transferability from wheat to barley is higher than that previously reported for EST-SSRs: 55.12% (Gupta et al., 2002), 55.8% (Zhang et al., 2005), and 53% (Yu et al., 2004). The differences are likely due to either sampling, since we restricted our comparisons to markers located on chromosome 2, or possibly the relaxed PCR

Table 5. Amplification profiles of polymorphic markers in four oat lines.

| Name | Sizes of amplicons† | | | | Name | Sizes of amplicons | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TAM-O-301 | Ogle 1040 | Kanota 156 | Ogle 157 | | TAM-O-301 | Ogle 1040 | Kanota 156 | Ogle 157 |
| Bmac0134 | 120, 300, 320 | 120, 240, 300, 320 | 120, 300, 320 | 120, 240, 300, 320 | Wmc506 | 180, 250, 500 | 250, 500 | 250, 500 | 250, 500 |
| Bmag0013 | 150, 220 | 150 | 150 | 150 | Gwm44 | 120, 420 | 120, 160, 420 | 280, 380, 400 | 280, 380, 400 |
| Bmac0306 | 200, 320 | 200, 240, 300 | 200, 240, 300 | 200, 240, 300 | Barc8 | 220, 260, 270 | 220, 260 | 260, 270 | 260, 270 |
| Bmag0173 | 700 | 300 | 300 | 300 | Barc187 | 250 | – | 250 | – |
| Bmag0344c | 120, 700 | – | 120, 700 | – | Barc101 | 400, 500 | 400, 500 | 400 | 500 |
| EBmac0405 | 120, 180 | 100, 120, 180, 310 | 120, 180 | 100, 120, 180, 310 | Wmc623 | 150, 200, 550 | 150, 200, 550 | 200, 550 | 150, 200, 550 |
| GMS021 | 270, 290, 310, 320, 500, 700 | 200, 290, 310, 320 | 290, 310, 320, 500 | 200, 290, 310, 320, 500, 700 | Barc87 | 180, 550 | 180, 320, 550 | 180, 550 | 180, 320, 550 |
| Bmac213 | 120, 290 | 120, 290, 400 | 120, 290 | 120, 290 | Wmc307 | 260 | – | 260 | – |
| cMWG660 | 550, 700, 720 | 700, 720 | 550, 700, 720 | 700, 720 | Wmc625 | 140, 150, 310 | 150, 310 | 150, 310 | 150, 310 |
| HVM54 | 350, 550 | 550 | 550 | 550 | Barc109 | 350, 800 | 350 | 350, 800 | 180, 350 |
| HVM62 | 180, 210, 700 | 210, 700 | 180, 210, 700 | 210, 700 | Wmc125 | 120 | 350 | 120 | 100, 250 |
| HVCSG | 200 | 200, 250 | 200 | 200, 250 | Barc20 | 315 | 310 | 320 | 310, 315 |
| Bmag0603 | – | – | 220, 400, 450 | 200, 460 | Barc59 | 160 | 200, 400 | 160 | 160, 400 |
| Bmag122 | 320, 450, 470 | 420, 470 | 420, 470 | 320, 470 | Wmc389 | 360 | 250, 360 | 360 | 250, 360 |
| HvOLE | 360 | 320, 360 | 320 | 320, 360 | Cfd13 | 215, 245, 395 | 410 | 215, 245, 395 | 410 |
| HVMLOH1A | 400, 600 | 600 | 600 | 600 | Barc134 | 120, 180 | 120 | 120, 180 | 120 |
| Bmac577 | 400, 550 | 265, 400, 550 | 400, 550 | 265, 400, 550 | Wmc419 | 70, 210 | 210, 240, 420 | 210 | 210, 240, 420 |
| Bmag375 | 150, 280, 300 | 150, 280, 300 | 500, 550, 700 | 500, 700 | Gwm43 | 400, 460 | 400, 460 | 400, 460, 470 | 400, 460 |
| Bmag387 | 250, 500, 600 | 250 | 250, 500, 600 | 250 | Barc85 | 200, 280 | 280, 360 | 200, 280, 360 | 280, 360 |
| EBmatc3 | 180 | 180, 260 | 180, 260 | 180, 260, 320 | Barc83 | 85, 180 | 50, 60, 180 | 85, 180 | 50, 50, 180 |
| Bmag5 | 600 | 150, 600 | 600 | 150, 600 | Wmc296 | 250, 360 | 360 | 360 | – |
| Bmag337 | 320, 350, 400 | 250, 350, 400 | 320, 350, 400 | 250, 350, 400 | Wmc382 | 220, 380 | 160, 220, 380 | 160, 220, 380 | 160, 220, 380 |
| Bmac18 | 150, 250, 270, 280 | 150, 270 | 150, 270 | 150, 270 | Wmc109 | 100, 180, 250 | 100, 250 | 100, 250 | 100, 250 |
| EBmac674 | 150, 270, 290, 300, 700 | 150, 270, 300, 700 | 150, 270, 290, 300, 700 | 150, 270, 300, 700 | Barc45 | 400 | 150, 400 | 400 | – |
| EBmac853 | 200, 280, 300, 500 | 200, 280, 300, 500 | 200, 280, 300, 500 | 300, 320, 500 | Wmc338 | 750 | 750 | 700 | 750 |
| Bmag11 | 280, 300, 500 | 280, 500 | 280, 300, 500 | 280, 300, 500 | Barc141 | 120, 310, 320 | 310 | 310, 320 | 310 |
| Bmag607 | 100, 200 | 200 | 100, 200 | 200 | Gdm109 | 190, 240, 260 | 190, 200, 240, 260 | 190, 240, 260 | 190, 200, 240, 260 |
| Wmc732 | 200, 280 | 200, 280, 300 | 200, 280, 300 | 200, 280, 300 | Wmc254 | – | – | 250 | – |
| Cfd2 | – | 220 | – | 220 | Wmc580 | 100, 200 | 100 | 100, 200 | 100 |
| Wmc470 | – | 280 | 280 | 300 | Wmc417 | 120, 280 | 120, 280 | 120 | 120, 280 |
| Wmc552 | 150 | 150, 220 | 150, 220 | 220 | Wmc158 | 140 | 160 | – | 160, 170 |
| Wmc95 | 160, 420 | 420 | 160, 420 | 420 | Gwm260 | 180, 320, 360 | 90 | 90 | 90 |
| | | | | | Wmc633 | 250, 300, 600 | 250, 300, 600 | 250, 300, 500 | 250, 300 |

†Sizes of the polymerase chain reaction products are estimated based on the 100-bp DNA marker.

conditions. Another important reason for the higher amplification frequency in our study is the lower annealing temperature used. Nevertheless, our results indicated the close genomic relationship between wheat and barley. There are few studies of the transferability of PCR-based markers from other species to oat. One study reported 12% of the SSR primer pairs tested from ryegrass transferred to oat (Jones et al., 2001). Based on results from the present study, markers from wheat seem more transferable to the oat genome than the ones from barley or ryegrass, although higher stringency conditions were used in the ryegrass study.

The transferability of markers from a donor genome depends on sequence conservation in specific regions of the recipient genomes where the primers are located. Our study showed that transferability rates might vary in different regions within the same donor genome. For example, amplification rates of SSR primers from the long arms of wheat chromosomes 4D and 6A appeared higher than those from the long arms of 1B and 7B (Fig. 2). For the practical purpose of enriching PCR-based markers in oat, the transfer rates from wheat and barley are acceptable and may be further improved if we can confirm that some subgenomes have higher transfer frequencies, and then select SSR primer pairs for testing based on this information. Despite some possible difference in transferability frequency for barley and wheat markers, the large number of available SSR primer sequences from these two species is a rich resource for oat marker generation.

In addition to genomic SSR markers, EST-derived SSR markers from other species may be another useful source for oat PCR-based marker development. In general, EST-SSR markers have a higher transferability across species than do genomic markers, while genomic SSR markers are more polymorphic. EST-SSR markers originate in coding sequences that are more conserved during evolution compared to genomic sequences. Direct comparisons of polymorphic rates between genomic SSR detected polymorphisms between parental lines of two oat mapping populations and 14 of 15 produced expected segregation ratios in the OT mapping population. The results of this study illustrate that wheat and barley are good genetic resources for marker development in oat. The results also point to the potential for future utilization in oat of other types of DNA markers, such as single nucleotide polymorphisms, from barley and wheat.

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